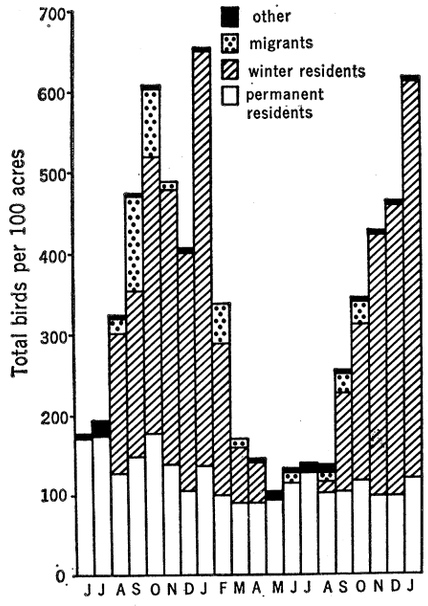

Fig. 2. Monthly average of bird populations estimated from counts per visit at Vero Beach locality (June 1966 to January 1968). Data exclude local birds visiting the site only by day. Solid black bar represents summer residents and estimates of less than ten migrants or winter residents.

at Tampa and frequently served as hosts, but they were uncommon at Vero Beach, where the larger armadillo population replaced them as one of the main sources of mammalian blood.

When seasonal changes in the relative abundance of birds and mammals are considered collectively (for each vertebrate class) there is still no evidence to explain the phenomenon adequately. A combination of avian census (*5*) and mammal trap (*6*) techniques provided monthly data on 1966 and 1967 populations at Vero Beach. Seasonal changes in the vertebrate populations were found, but these changes do not parallel the seasonal changes in the ratio of feeding on birds to that on mammals in *C. nigripalpus*. For example, wild bird populations increase greatly during migrations and certain periods of the winter (Fig. 2). The winter residents undoubtedly contribute to the avian feedings during this period, but they start to arrive in August and September (when feeding on mammals remains high) and they depart in February and March, at least 2 months before the decrease in feeding on birds. Likewise, no increase in feeding on birds occurred during the migration periods. This might be expected, however, since migrating birds are transient, usually flying at night (when *Culex* feed) and foraging and resting during the day (*7*).

If seasonal change in host availability (due to change in host numbers, behavior, age, and so forth) is eliminated as the primary cause for seasonal change in mosquito feeding, we are left with factors that directly or indirectly affect the behavior of the mosquito population. Present evidence suggests at least two possibilities. Periods of maximum feeding on mammals closely parallel the periods of peak abundance of mosquitoes. This is true for *C. tarsalis* as well and suggests that population size might have an important influence on mosquito behavior. Second, seasonal feeding patterns are closely related to the seasonal weather conditions, indicating that temperature and atmospheric moisture might influence the mosquito's feeding behavior or its ability to find one class of hosts more readily than the other. A combination of factors may be at play although the remarkable similarity in patterns between the two species in several biotically different localities would favor some factor common to all.

To be both the enzootic and epidemic vector (dual vector) of a virus enzootic in birds (such as St. Louis and western encephalitis), a mosquito must have a varied feeding pattern. Both *C. nigripalpus* and *C. tarsalis* meet this requirement, and both are efficient vectors of arbovirus. Evidence that *C. tarsalis* shifts from primarily avian hosts in the spring (probable enzootic period of virus buildup) to a high proportion of mammalian hosts in the summer and fall (epizootic and epidemic season), when considered with the similar finding in *C. nigripalpus*, strongly favors this "change in hosts" as a basic requirement of a dual vector.

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Raman Spectra of Crystalline Lysozyme, Pepsin, and Alpha Chymotrypsin

Abstract. *Satisfactory Raman spectra of crystalline lysozyme, pepsin, and alpha chymotrypsin were obtained with laser excitation. The spectra are very similar to each other, but show enough minor differences to make this a useful method of identification. The readily identified bands assignable to specific groupings are noted.*

The development of improved Raman spectrometers (*1*) assembled with ion-laser sources, double monochromators, and pulse-counting detection makes it possible to record the Raman spectra of many crystalline hormones and enzymes with good signal-to-noise ratios. Even though a strong fluorescence background is present in these materials, conditions can be adjusted to bring out the Raman lines (*2*).

I now report on the Raman spectra

Table 1. Observed Raman lines in enzymes. No Raman lines were observed between 30 and 500 cm^{-1}.

α-Chymotrypsin (cm^{-1})	Lysozyme (cm^{-1})	Pepsin (cm^{-1})
~3300	~3300	~3300 NH
3062	3053	3064 =CH
2968	2964	2967 CH
2936	2926	2935 CH
2875	2870	2885 CH
1667	1667	1674 amide C=O
1619	1625	1614 C=C
1578	1591	
1551	1512	1554 amide NH
1453	1430	1455 CH
1358	1356	1341
1341	1336	1313
1245	1251	1250
	1202	1207
		1177
		1156
1127	1050–1139	1126
1034	1011	1032
1007	1003	1006
976		
878	900	879
855	876	856
830		831
758	768	760
644		645
627		623
568		
512	505–595	521

of the crystalline enzymes lysozyme (*3*), alpha chymotrypsin (*4*), and pepsin (*5*). The spectra were recorded with a Raman spectrometer built from a Spex-1400 double monochromator, an I.T.T. FW-130 "Star Tracker" phototube (S-20 surface) held at −30°C and an EG & G pulse-counting system. The pulses were integrated with an RC (resistance-capacitance) circuit. The integrated signal was passed through an impedance-matching Keithley electrometer to a Mosely recorder. Simultaneously, the output from the pulse-counting-circuit discriminator was displayed on a scaler. The typical dark-count rate, with the pulse-counting-circuit discriminator set at zero, was two pulses per second.

The samples, in the form of a compressed crystal powder, were front illuminated (*2*) with a laser beam focused by a 6-cm focal-length lens. Although 0.25-g samples were used in these preliminary experiments, the illuminated volume was about 10^{-5} cm^3. There seems to be no reason why samples of 10^{-6} g could not be successfully used.

The spectrum of lysozyme was obtained with an 80-mw helium-neon laser. The spectra of α-chymotrypsin and pepsin were recorded with 250 mw in the 5145-Å argon line, with the use of a laser. At least for the few materials which I was able to examine with argon-laser excitation, fluorescence was not markedly worse than the helium-neon excitation. The signal-to-noise ratios, were, for the same running conditions, a factor of 5 better with 250 mw in the 5145-Å argon line than with 80 mw in the 6328-Å He-Ne line.

The spectra were all recorded with a 4-cm^{-1} spectral bandpass, at a speed of about 10 cm^{-1} per minute. In view of the width of the observed lines, the spectra, at least with argon-laser excitation, probably could have been recorded much faster. If the entrance-slit width is to be filled, the diameter of the focused laser beam limits the spectral bandpass to a maximum of about 5 cm^{-1}.

The spectra obtained are listed in Table 1 and in Fig. 1. In the region 2800 to 3500 cm^{-1}, only the spectrum of pepsin is shown, because the spectra were almost identical in this region. No verifiable Raman lines were observed below about 500 cm^{-1}, although all the spectra were examined, with narrowed slits, down to 30 cm^{-1}. A line observed near 230 cm^{-1} in all of the spectra (this line is marked *a* in the spectrum of pepsin) is believed to be a grating ghost.

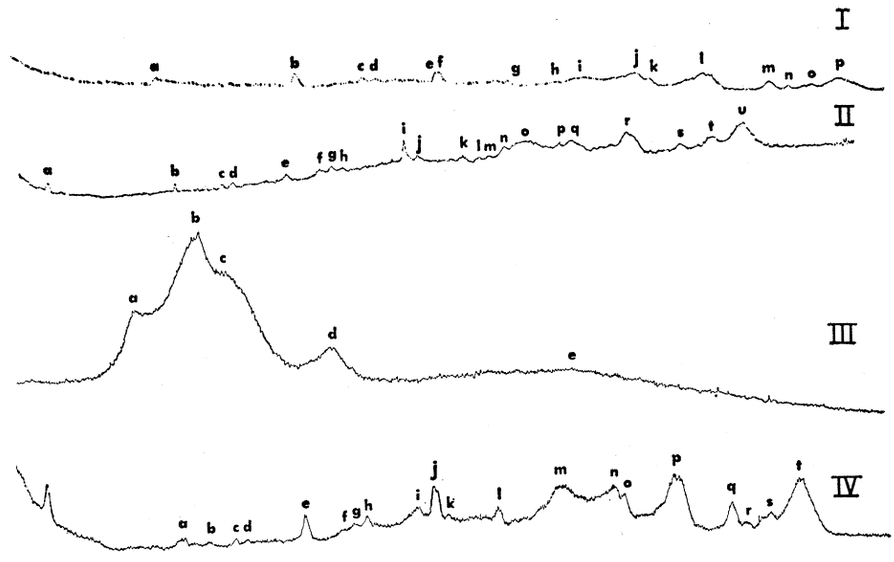

Fig. 1. (Spectrum I) Raman spectrum of crystalline lysozyme. Helium-neon 6328 Å excitation; line positions (cm^{-1}): *a*, 505; *b*, 768; *c*, 876; *d*, 900; *e*, 1003; *f*, 1011; *g*, 1139; *h*, 1202; *i*, 1251; *j*, 1336; *k*, 1356; *l*, 1430; *m*, 1512; *n*, 1591; *o*, 1625; *p*, 1667. (Spectrum II) Raman spectrum of crystalline pepsin. Argon 5145 Å excitation. Line positions (cm^{-1}): *a*, 239; *b*, 521; *c*, 623; *d*, 645; *e*, 760; *f*, 831; *g*, 856; *h*, 879; *i*, 1006; *j*, 1032; *k*, 1126; *l*, 1156; *m*, 1177; *n*, 1207; *o*, 1250; *p*, 1313; *q*, 1341; *r*, 1455; *s*, 1554; *t*, 1614; *u*, 1674. (Spectrum III) Raman spectrum of crystalline pepsin. 2800 to 3500 cm^{-1}. Argon 5145 Å excitation. Line positions (cm^{-1}): *a*, 2885; *b*, 2935; *c*, 2967; *d*, 3064; *e*, 3289. (Spectrum IV) Raman spectrum of crystalline α-chymotrypsin. Argon 5145 Å excitation. Line positions (cm^{-1}): *a*, 512; *b*, 568; *c*, 627; *d*, 644; *e*, 758; *f*, 830; *g*, 855; *h*, 878; *i*, 976; *j*, 1007; *k*, 1034; *l*, 1127; *m*, 1245; *n*, 1341; *o*, 1358; *p*, 1453; *q*, 1551; *r*, 1578; *s*, 1619; *t*, 1667.

Even in the absence of the fluorescence background, the enzymes would not be ideal specimens for Raman spectroscopy. They are weak scatterers and tend to have extremely broad lines. The width of the lines depends on two factors. The first is hydrogen bonding, which manifests itself in the width of the NH-stretching modes near 3300 cm^{-1}. The major cause of the line width, extending over several hundred wave numbers in some cases, is sheer size of the enzyme molecules. If the enzymes were, in zeroth order, considered as mere superpositions of the 20 basic amino acids, their spectra from 500 cm^{-1} to 1200 cm^{-1} would be nearly continuous (*6*). The situation is different from that of crystalline polymers, where the repeat unit is generally small and the selection rules forbid spectroscopic activity in most of the normal modes (*7*).

The spectra in the CH- and NH-stretching regions are remarkably similar and give little information. The amide bands and the C=C stretching bands differ slightly. The remainder of the observed bands differ enough so that the Raman spectra will be useful as a method of identification as soon as a library of spectra has been built up.

In working with crystal powders, the Raman spectrum has several advantages over the infrared spectrum. (i) The whole spectrum from 30 to 3300 cm^{-1} is exhibited. (ii) The method is not destructive. Preparation of Nujol mulls or KBr disks is not necessary. (iii) Samples as small as a few micrograms may be used.

Raman spectroscopy lends itself readily to the study of aqueous solutions. With our samples, the low solubility of proteins is expected to be a difficulty.

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